is an upper respiratory disease. Experimentally produced upper respiratory illness with this virus has been achieved by nasopharyngeal inoculation (3), by deposition of virus on selected sites in the upper respiratory tract, and by inhalation of large particle aerosols (4), which are primarily deposited in the upper respiratory tract.

In a series of experiments, Buckland and his associates circumvented the problem of precise location of deposition of airborne particulates by direct application of coxsackievirus A-21 to specific locations in the upper respiratory tract. Their findings showed the nasal mucosa to be exquisitely susceptible to infection, whereas the oropharynx and nasopharynx were refractory to doses several orders of magnitude greater. In subsequent studies, volunteers were infected with doses comparable to those directly instilled when presented in relatively large airborne particles, virtually all of which might be expected to be deposited on the nasal mucosa. These authors concluded that only particles retained in the upper respiratory tract are of significance in transmission of naturally occurring disease.

In attributing production of upper respiratory disease to the small particles generated with the Collison atomizer, Gerone and his associates have not rigorously excluded the contribution of that portion of the particles larger than 2 μ, which might be expected to be retained in the upper respiratory tract. From analysis of the particle size spectrum of the aerosol, approximately one-fifteenth the dose presented might be so retained (5). This may well be a significant quantity of virus, of itself capable of initiating infection.

Further experimentation, either by use of aerosols whose upper respiratory retention is negligible, or by bypassing the upper respiratory tract via an artificial airway, are needed if this matter is to be definitively resolved.

Most disappointing to this reviewer is the lack of information presented upon the airborne stability of coxsackievirus A-21 under varying conditions of relative humidity and temperature. The observations of Buckland and his associates indicate a biological decay rate of 50% per min for virus in small particles and roughly 25% per min in the larger particles, if decay is linear. Such values are compatible with droplet infection. Far greater airborne stability is required for significant airborne transmission under ordinary conditions. Valuable information could be obtained by sequential examination of static aerosols with slit samplers or impingers.

In summary, the authors have described an aerosol used to induce infection in man. This discussant believes that further, more critical examination is required to definitively establish the significance of deep respiratory deposition of small particles in production of upper respiratory disease, and hence the appropriateness of the model for the study of naturally acquired infection. It is hoped that further studies will clarify this. Similarly, improvements in high-volume sampling, combined with knowlege of airborne stability of this virus, will permit more critical evaluation of the role of airborne dissemination in coxsackievirus A-21 upper respiratory disease.

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Author's Comments on the Discussion

P. J. GERONE, R. B. COUCH, R. G. DOUGLAS, AND V. KNIGHT

In Col. Gochenour's discussion of our paper, several points were made with which we are in complete agreement. Other issues were raised, however, regarding which we would like to clarify the position or the conclusions that have been reached.

In regard to the large volume air sampler (LVS), the discussant has outlined its limitations as being (i) excessive evaporative loss of collecting fluid, (ii) inability to estimate particle size distribution, and (iii) its failure to "meaningfully quantitate airborne virus." The loss of fluid by evaporation in the LVS has not been a serious problem. When known concentrations of coxsackievirus A-21 were added to the collecting medium and circulated through the LVS for periods three times longer than the sampling periods described in the paper, no virus loss occurred. On the contrary, when low-concentration aerosols were sampled, the reduced volume of fluid that had to be tested served as an advantage.

The LVS was not designed to measure particle sizes of the aerosols it samples; furthermore, no other equipment is available which can analyze particle size and, yet, handle these large volumes of air.

Under the conditions in which the LVS was tested, it was found to be a quantitative sampler. The data in the last figure of the paper were replotted (Fig. 1a) to show the relationship between virus concentration recovered and virus concentration in the room air. It is readily apparent not only that a relationship does exist, but that there is a direct proportionality between the amount of airborne virus in the room and the quantities recovered in the LVS.

The studies comparing the LVS and all glass impinger (AGI; Table 5) raised a question in the discussant's mind regarding the variability seen in virus recoveries and the consistency demonstrated in the fluorescein recoveries. It should be stated that two variables were present in the virus determinations which were not present in the fluorescein assays. These are: (i) the biological inactivation of the virus and (ii) the sensitivity of the cell cultures used in the assay procedure. The four virus recovery values with the LVS in actual $TCID_{50}$ ranged from 4.6 to 5.1 $\log_{10}$, and are consistent with the 0.25 $\log_{10}$ standard deviation of the assay procedure. This standard deviation, however, cannot be applied to the virus values obtained with the AGI, because those end points were calculated by the Fischer-Yates dilution technique. Despite the limitations imposed by this assay procedure, the mean recovery in the LVS and AGI were remarkably similar.

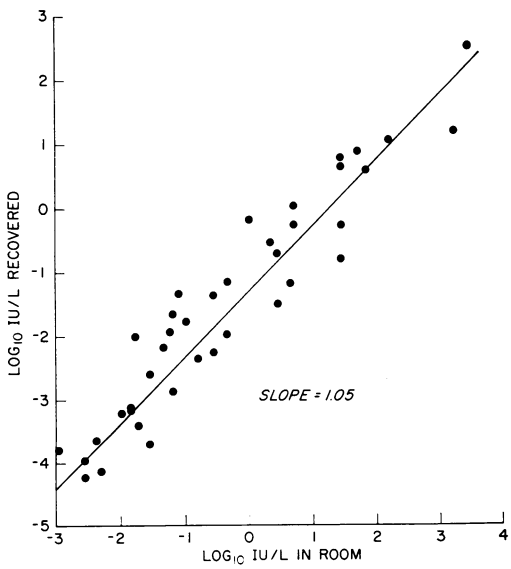

FIG. 1a. *Relationship of coxsackievirus A-21 concentrations in room air and quantities recovered by the large volume air sampler.*

TABLE 1a. *Coxsackievirus A-21 aerosols used in volunteer inoculations*

Run no.	Dilution of inoculum pool	Suspension concn ($\log_{10}$ $TCID_{50}$ per liter)	Aerosol concn ($\log_{10}$ $TCID_{50}$ per liter)	
			Predicted	Actual
1	$10^{-4.0}$	8.0	1.74	2.08
2	$10^{-4.5}$	7.5	1.30	0.35
3	$10^{-4.5}$	7.5	1.30	1.24
4	$10^{-4.5}$	7.5	1.30	1.42
5	$10^{-4.5}$	7.5	1.30	1.42
6	$10^{-4.6}$	7.4	1.22	1.11

The discussant also questioned the predictability of doses administered to volunteers with the experimental aerosols. We agree with his enumeration of the factors which may influence predictability. Undue emphasis, however, was placed on a few points which strayed from the line shown in Fig. 1 of the manuscript. The maximal deviation between the predicted and actual determination was 1.1 $\log_{10}$, and only 3 of the 27 points plotted on the graph (1 in 9 determinations) were more than 0.5 $\log_{10}$ from the predicted values. These results have been interpreted by the authors as representing good predictability for aerosol inoculations with *this virus*. This can be further supported by actual figures, shown in Table 1a, taken from the subsequent volunteer experiments. Five of the six predicted values were within 0.35 $\log_{10}$ from the actual determinations and four of these were within 0.15 $\log_{10}$ of the anticipated concentration.

The two main points that were established by the studies of sneezes and coughs were: (i) these expiratory events produce large numbers of small aerosol particles capable of remaining airborne for long periods of time, and (ii) sufficient quanti-

ties of coxsackievirus A-21 are present in these particles to induce infection in susceptible subjects. Additionally, it should be noted that most of the particles produced by sneezes and coughs are in a size range comparable to those generated by the Collison atomizer; however, because of the presence of small numbers of large particles in sneezes and coughs, the volume distributions of the natural and experimental aerosols are different. The distribution of virus according to number or volume of particles in natural aerosols has not been determined. The occurrence of airborne virus in cough specimens was found to be statistically related to the quantities of virus in the nasal and oral secretions (1).

The discussant's observation that the larger particles in the experimental aerosol may have initiated upper respiratory infection and illness in the volunteers may be valid, and was recognized by us in a previous report (2).

On the basis of the discussant's comments regarding types of clinical illness produced with coxsackievirus A-21 infections, additional clarification of our findings is necessary. The predominant clinical response produced by this virus is upper respiratory illness, regardless of whether he infection occurs in natural circumstances or follows experimental inoculation by nasal instillation, large-particle aerosols, or *small-particle* aerosols. With one strain (49889 HEK_1), however, lower respiratory illness was the predominant response and occurred only after small-particle aerosol inoculation. There appears to be no doubt that the upper respiratory passages are extremely susceptible to infection with this virus, and we agree that deposition at this site may be responsible for the consistent finding of upper respiratory illness in natural and experimentally induced disease. Finally, as stated in the previous paper (1), the question of how this virus is transmitted in nature has, at the present time, not been answered in this laboratory or elsewhere.

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